

We Claim:

1. An *in vitro* system comprising a Notch ligand that supports T cell lymphopoiesis but does not support B cell lymphopoiesis.  
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2. An *in vitro* system of claim 1 comprising a Notch ligand that induces T cell lineage commitment and differentiation, stage-specific progenitor expansion, TCR gene rearrangement, and T cell differentiation by hematopoietic progenitors and embryonic stem cells in the absence of the thymus.  
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3. An *in vitro* system of claim 1 comprising a cell preparation comprising cells that express a Notch ligand.
4. An *in vitro* system of claim 3 that induces TCR V(D)J rearrangement, and T cell  
15 differentiation by hematopoietic progenitor cells or embryonic stem cells.
5. An *in vitro* system as claimed in claim 3 wherein the cell preparation comprises stromal cells that express a Notch ligand.
- 20 6. An *in vitro* system as claimed in claim 5 wherein the Notch ligand is a member of the Delta family.
7. An *in vitro* system as claimed in claim 6 wherein the Notch ligand is Delta-like 1 or Delta-like-4.  
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8. An *in vitro* system as claims in claim 3 wherein the cells lack functional macrophage colony stimulating factor (M-CSF).
9. An *in vitro* system as claimed in claim 5 wherein the stromal cells are OP9 cells.

10. An *in vitro* system as claimed in claim 9 wherein the OP9 cells comprise a Delta-like-1 nucleic acid sequence shown in SEQ ID NO:8 or SEQ ID NO:9.
- 5 11. An *in vitro* system as claimed in claim 9 wherein the OP9 cells comprise a Delta-like-4 nucleic acid sequence shown in SEQ ID NO:10 or SEQ ID NO:11.
12. A method of forming cells of the T cell lineage comprising culturing cells that are capable of differentiating into cells of the T cell lineage with an *in vitro* system of claim  
10 1 to form cells of the T cell lineage.
13. A method according to claim 12 wherein the cells that are capable of differentiating into cells of the T lineage are selected from hematopoietic progenitor cells, hematopoietic stem cells and embryonic stem cells.
- 15 14. A method of claim 12 further comprising separating the cells of the T cell lineage to obtain populations of cells largely consisting of one or more types of cells of the T cell lineage.
- 20 15. A method of claim 14 wherein the population of cells that is separated comprises immature T cells.
16. A method of claim 14 further comprising inducing the immature T cells to form mature T cells.
- 25 17. A method of claim 14 wherein the population of cells are formulated in a pharmaceutically acceptable carrier, auxiliary or excipient.
18. A T cell lineage composition comprising cells of the T cell lineage generated with

a system as claimed in claim 1 .

19. A T cell lineage composition produced by culturing cells capable of differentiating into cells of the T cell lineage with a system of claim 1 and isolating cells of the T cell lineage in the culture.

20. A T cell lineage composition of claim 19 comprising one or more of:

(a) progenitor or precursor cells committed to the T cell lineage;

(b)  $CD4^- CD8^- CD25^+ CD44^+$ ;

10 (c) cells that have undergone CD4 or CD8 lineage commitment;

(d) precursor thymocytes that are  $CD4^+ CD8^+$  double positive (DP);

(e) single positive cells that are  $CD4^+ CD8^+$  or  $CD4^+ CD8^-$  and optionally  $TCR^{hi}$ ;

(f)  $TCR-\alpha\beta^+$  and/or  $TCR-\gamma\delta^+$  T cells;

(g)  $CD3^+ CD90^+$ ; and

15 (h) mature and functional T cells characterized as  $TCR/CD3^{high} CD4^- CD8^+$  or  $CD4^+ CD8^-$ .

21. A composition which comprises a nutrient medium that has been conditioned by exposure to a Notch ligand cell preparation that supports T cell lymphopoiesis but does not support B cell lymphopoiesis.

22. A method for expanding cells of the T cell lineage comprising (a) culturing cells capable of differentiating into cells of the T cell lineage with a system of claim 1; and (b) isolating increased numbers of cells of the T cell lineage.

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23. An expanded cellular composition comprising cells of the T cell lineage obtained by a method of claim 22.

24. A method as claimed in claim 23 wherein the number of cells is increased by at

least about 10 to 15 fold.

25. A pharmaceutical composition comprising cells of the T cell lineage generated with a system of claim 1 and a pharmaceutically acceptable carrier, excipient, or diluent.

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26. A method for screening for modulators of cells of the T cell lineage comprising the steps of:

(a) generating cells of the T cell lineage with a system as claimed in claim 1 in the presence of a test substance; and

10 (b) detecting the presence or absence of an effect of the test substance on the survival of the cells or on a morphological, functional or physiological characteristic and/or molecular biological property of said cells, whereby an effect altering cell survival, a morphological, functional, or physiological characteristic and/or a molecular biological property of the cells indicates the activity of the test substance.

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27. A method of treating a patient with a condition involving cells of the T cell lineage or requiring replacement of cells of the T cell lineage comprising transferring a T cell lineage composition as claimed claim 18 into the patient.

20 28. A method according to claim 27 to treat a patient with a T cell deficiency.